

Claims

1. A genetic construct which comprises:

- a. a positive selectable marker gene,
- b. a negative selectable marker gene, and
- c. direct repeats of a gene of interest that flank the positive and negative selectable marker genes of (a) and (b).

2. The genetic construct of Claim 1 wherein the negative selectable marker gene is *CodA*.

3. The genetic construct of Claim 2 wherein the positive selectable marker gene is *NPTII*, *BAR*, *PAT* or *EPSP* synthase

4. A method of removing selectable marker genes from transformed eukaryotic cells which comprises:

- a. transforming cells with a genetic construct of Claim 1,
- b. culturing the cells of (a) on a positive selective medium,
- c. transferring the transformed cells in (b) onto a negative selective medium,

and

- d. selecting the cells that grow on the negative selective medium

wherein the selected cells that grow on the negative selective medium contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

5. The method of Claim 4 wherein the negative selectable marker gene is *CodA*.

6. The genetic construct of Claim 1, wherein said construct comprises a polynucleotide sequence in the 5' to 3' (right to left) direction:

- a. a gene sequence of interest,
- b. a positive selectable marker sequence,
- c. a negative selectable marker sequence and
- d. a repeat of the gene sequence of interest in (a) above.

7. The genetic construct of Claim 6 wherein the negative selectable marker sequence is *CodA*.

8. A method of removing selectable marker genes from transformed plant cells which comprises:

- a. transforming cells with a genetic construct of Claim 1 to form T0 transformants,
- b. culturing the cells of (a) on a positive selective medium,
- c. selecting the T0 transformant cells that grow on the positive selective medium,
- d. regenerating a fertile T0 plant from the T0 transformant cells whereby T1 seed is formed,
- e. collecting the T1 seed from the T0 plant or the seed from a subsequent Tn generation plant wherein n is a whole number greater than one,
- f. germinating the T1 seeds or Tn seeds on a negative selective medium to form seedlings, and
- g. selecting the seedlings that grow on the negative selective medium

wherein the selected seedlings contain the gene sequence of interest but neither the positive selectable marker sequence nor the negative selectable marker sequence.

9. The method of Claim 8 wherein the negative selectable marker gene is *CodA* and the negative selective medium comprises 5-fluorocytosine.

10. The genetic construct of Claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

GI-PS-NS-GI

wherein GI represents a gene of interest, PS represents a positive selectable marker gene and NS represents a negative selectable marker gene.

11. The genetic construct of Claim 10 wherein NS is *CodA*.

12. The genetic construct of Claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

GI-NS-PS-GI

wherein GI represents a gene of interest, NS represents a negative selectable marker gene, and PS represents a positive selectable marker gene.

13. The genetic construct of Claim 12 wherein NS is *CodA*.

14. The genetic construct of Claim 1, wherein said construct comprises, in the 5' to 3' direction (left to right), the formula:

AGx-GI-PS-NS-GI-AG'y

wherein AG and AG' represent additional genes of interest, x represents an integer of 1 or larger, y represents an integer of 0 or larger, GI represents a gene of interest, NS represents a negative selectable marker gene, and PS represents a positive selectable marker gene.

15. The genetic construct of Claim 14 wherein the genes represented by AG and AG' are never the same.

16. The genetic construct of Claim 14 wherein the NS is *CodA*.
17. The method of Claim 4 wherein the eukaryotic cell is a plant cell.
18. The method of Claim 4 wherein the plant cell a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell.
19. The method of Claim 8 wherein the plant cell is a monocot or dicot cell.
20. The method of Claim 19 wherein the plant cell a corn, soybean, cotton, wheat, canola, tobacco, Arabidopsis, rice, safflower or sunflower cell.